REMARKS

Claims 24-26, 29, 32, 34, 35 and 39-41 are cancelled above, without prejudice. Applicants reserve the right to pursue any subject matter affected by the foregoing amendments/cancellations in co-pending or later filed continuation or divisional applications. Upon entry of the foregoing amendments, claims 23, 27, 28, 30, 31, and 33 will be before the Examiner for consideration.

The Examiner maintains the object to Figures 3G-3K. In Applicants' previous response FIGs 3G-K were deleted, as these figures were not essential to describe or enable any embodiments of the subject invention, i.e., they are not essential to patentability. Applicants provide herewith a new drawing page which includes Figures 3C-F, with 3G-K removed. Applicants believe that this newly provided page addresses the pending objection. Reconsideration is requested.

The objection of claim 32 as pertaining to non-elected subject matter is moot in view of the cancellation of claim 32.

The rejection of claims 34 and 35 is moot in view of the cancellation of claims 34 and 35.

Claims 23-38, 30-35 and 39-41 are rejected under 35 USC § 112, first paragraph, as not meeting the written description requirement. Applicants assert that the amendments to claims 23 and 30 obviate this rejection. Accordingly, in view of the foregoing amendments and remarks, Applicants respectfully request the reconsideration and withdrawal of this 35 USC § 112 written description rejection.

Claims 23-38 are rejected under 35 USC § 112, first paragraph, as not being enabled. Applicants assert that the amendments to independent claims 23 and 30 obviate this rejection.

Accordingly, Applicants respectfully request the reconsideration and withdrawal of this 35 USC § 112 enablement rejection.

Claims 23-28 and 30-35 and claims 39-41 are rejected under 35 USC 112, second paragraph, as being indefinite. To the extent this rejection applies to claims 34 and 35, it is now moot in view of the cancellation of these claims. With respect to this rejection as it relates to the use of the designation P301L, Applicants assert that the amendments to claims 23 and 30 further clarify the aberrant form of tau being implemented. Claim 23 and 30 have been amended to further clarify that the gene used in the respective methods pertains to "a gene encoding an aberrant form of a human tau protein comprising the P301L mutation associated with FTDP-17. . . ." Thus, this discussion clarifies that it is the aberrant form of a tau gene that encodes for a protein having a proline at the 301 position. In view of these amendments and remarks, Applicants request reconsideration of this rejection.

Next, the Examiner rejects claims 30-33 and 40 on the basis of indefiniteness. The Examiner states that claim 30 is indefinite as the preamble recites that behavioral changes will occur but alleges that no behavioral changes are achieved. Claim 30 has been amended to recite that "somatically transferring a gene reduces memory in said living rat or mouse." Support for the reduction of memory in a rat or mouse through somatically transferring one or more genes into the brain of a rat or mouse is found on pages 18 and 19 of the subject application. In addition, as further support for the achievement of loss of memory, Applicants attach a poster from the 2004 Society of Neuroscience meeting pertaining to additional specific data involving the somatic transfer of a gene encoding an aberrant tau protein comprising the P301L mutation. This poster represents data showing that somatic transfer of such aberrant tau protein reduces memory in rats. In view of the foregoing remarks, Applicants respectfully request the reconsideration and withdrawal of the rejection on this basis.

Lastly, claims 34 and 35 were rejected as being anticipated by Gotz et al. This rejection is now moot in view of the cancellation of claims 34 and 35. Reconsideration is requested.

All grounds for rejection or objection having been addressed and overcome herein, it is respectfully urged that this application is in condition for allowance. Should the Examiner be of the opinion that there remain valid grounds on which any of the claims as herein amended may be rejected, it is respectfully requested that the undersigned be accorded the courtesy of a telephonic interview to address and overcome any such remaining grounds for rejection.

Respectfully submitted,

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Program Number: 787.1 Day / Time: Tuesday, Oct. 26, 1:00 PM - 2:00 PM

Modeling Alzheimer's tauopathy: Adeno - associated virus vector expressing mutated tau in the entorhinal cortex of rats impairs spatial working memory

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Entorhinal cortex degeneration begins very early in Alzheimer's disease (AD), a disorder characterized by severe memory disruption. Indeed, loss of entorhinal volume is predictive of AD and two of the hallmark neuroanatomical markers of AD, amyloid plaques and neurofibrillary tangles (NFTs), are particularly prevalent in the entorhinal area of AD-afflicted brains. Gene transfer techniques were used to create an Alzheimer's model by injecting a recombinant adeno-associated viral vector with a mutated human tau gene (P301L) into the entorhinal cortex of adult rats. The objective of the present investigation was to determine whether localized expression of human mutated tau would produce either behavioral or anatomical pathology. Spatial memory on a Y-maze was tested for approximately four months post-surgery. Upon completion of behavioral testing the brains were assessed for expression of human tau and evidence of tauopathy. Rats injected with the tau vector became persistently impaired on the task after about 30 days of postoperative testing, whereas the control rats injected with a green fluorescent protein control vector performed at criterion levels during that period. Histological analysis confirmed the presence of hyperphosphorylated tau and NFTs in the entorhinal cortex and neighboring retrohippocampal areas as well as degeneration of the perforant path. Thus, vector-induced tauopathy in retrohippocampal areas significantly impaired mnemonic functioning in rats.

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Modeling Alzheimer's tauopathy: Adeno-associated virus vector expressing mutated tau in the entorhinal cortex of rats impairs spatial working memory

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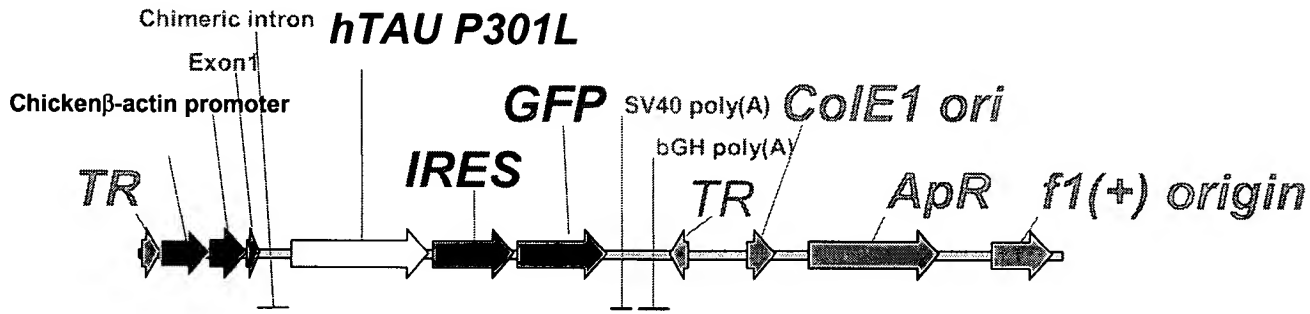
Introduction

- Neuronal loss in the entorhinal cortex and surrounding areas is particularly prevalent in the early stages of Alzheimer's disease (AD) (reviewed in Van Hoesen, Hyman, Damasio, 1991).
- This neuronal loss is associated with the formation of amyloid plaques and neurofibrillary tangles (NFTs), which constitute the major pathological hallmarks of the disease.
- Tau is a key player in the formation of NFTs and other tauopathies, including pretangles and neuropil threads. Some isoforms of tau have proven to be more pathogenic than others. Due to abnormal phosphorylation, the P301L gene has been implicated in AD and other progressive neurodegenerative disorders (Klein *et al.*, 2004, and Adamec *et al.*, 2002 and Arendash *et al.*, 2004)

The P301L rAAV Construct

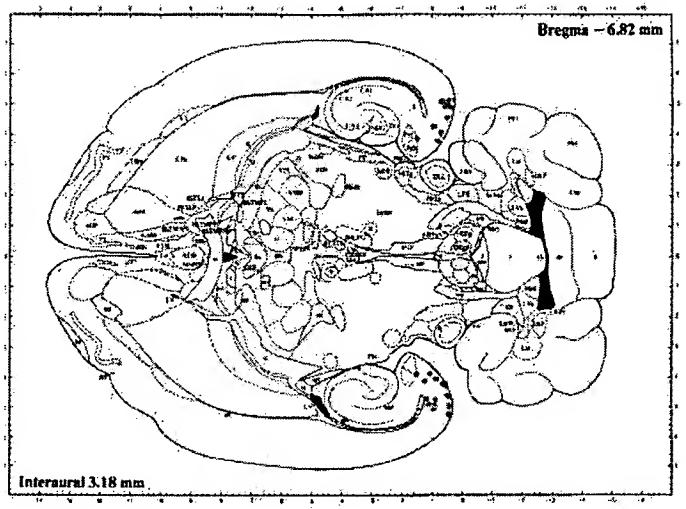
- Gene transfer techniques were used to create a model of Alzheimer's disease in rat.
- A recombinant adeno-associated virus (rAAV) vector was used for gene transfer, containing either GFP or P301L (abnormal tau).
- The rAAV vector has been shown to produce tau-immunoreactive neuronal lesions within one month *in situ* (Klein *et al.*, 2004).

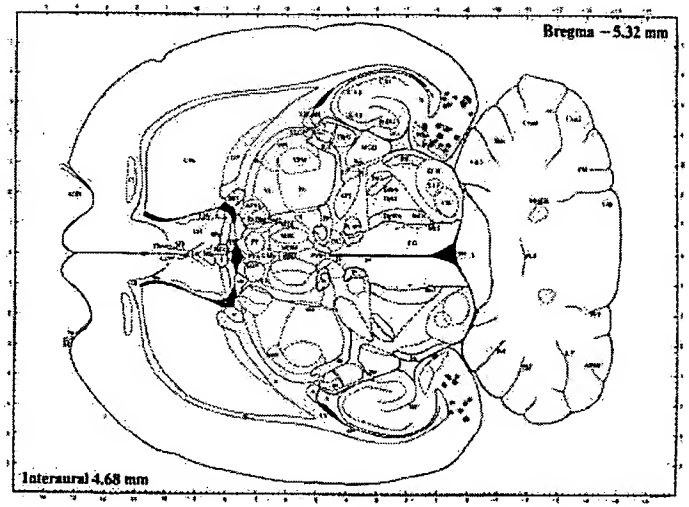
AAV2-P301L tau (FTDP-17)



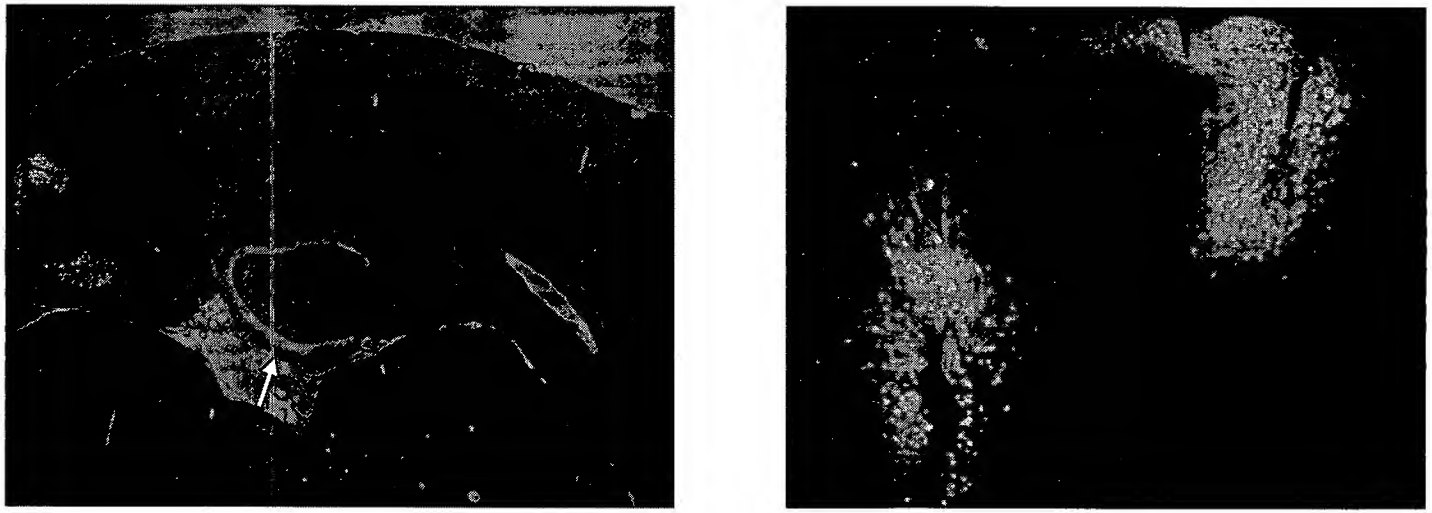
Injection Site and Behavioral Testing

- rAAV virus vector was injected bilaterally into the entorhinal and neighboring retrohippocampal areas of 20 male Sprague-Dawley rats.
- Spatial memory was tested using a reinforced alternation task with a 40s intertrial interval on a Y-maze.
- Animals were allowed to recover for seven days after surgery before behavioral testing was initiated.
- The ability of the rats to acquire and retain the learned alternation task was tested for approximately four months after surgery.





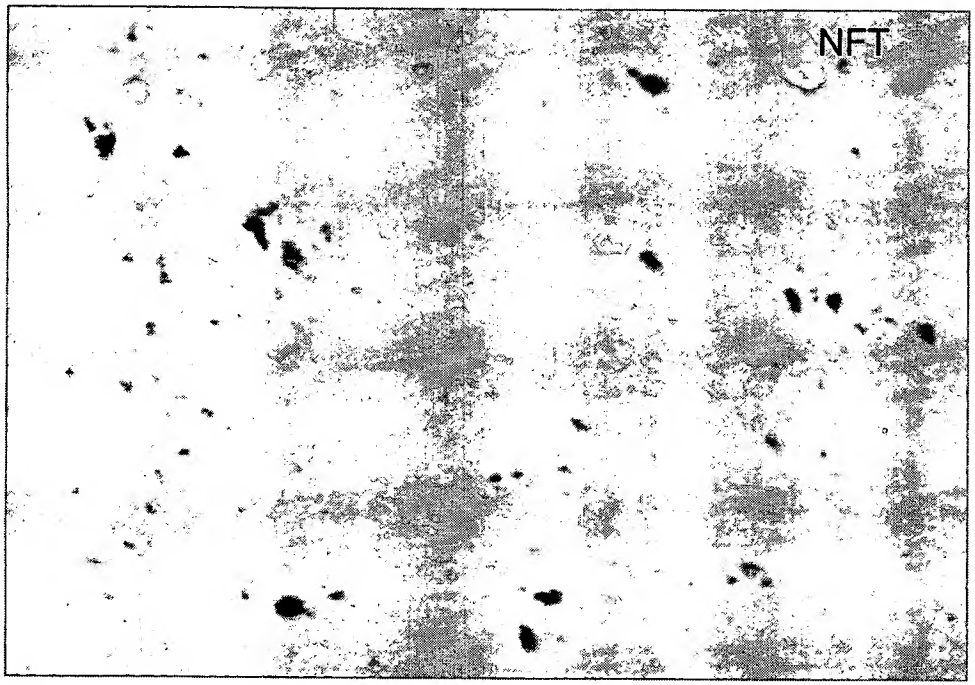
Histological Results

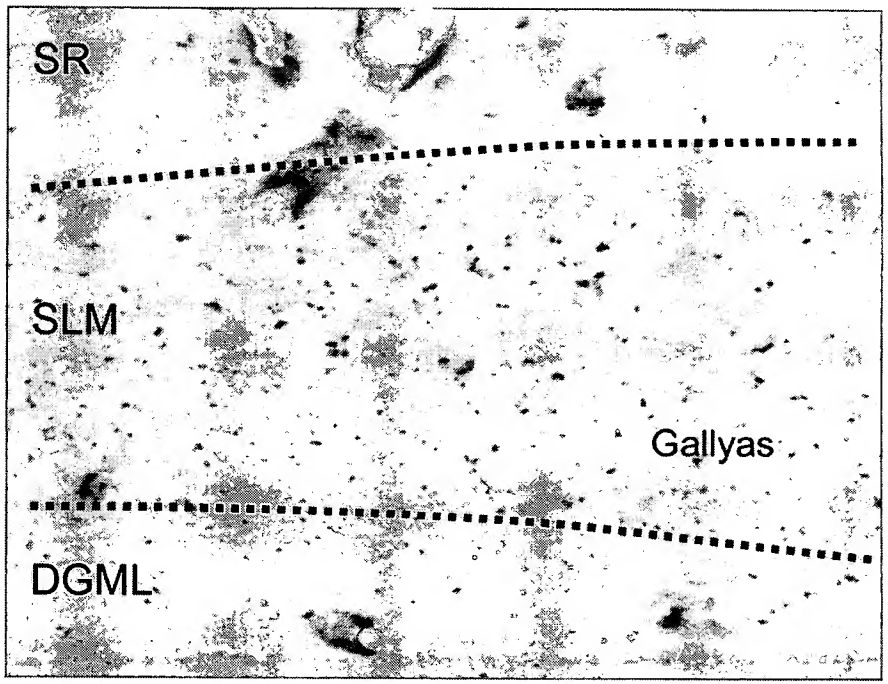


- Control rAAV (constructed to drive the expression of GFP) injection led to a robust expression in layers II and III of the entorhinal cortex, with efferents projecting to the dentate gyrus via the perforant path (white arrow, left panel).

Histological Results

- Tissue was positive for NFTs in the entorhinal cortex near the injection sites, as shown in the bottom left panel.
- Gallyas staining, bottom right panel, confirmed degeneration in the hippocampus.

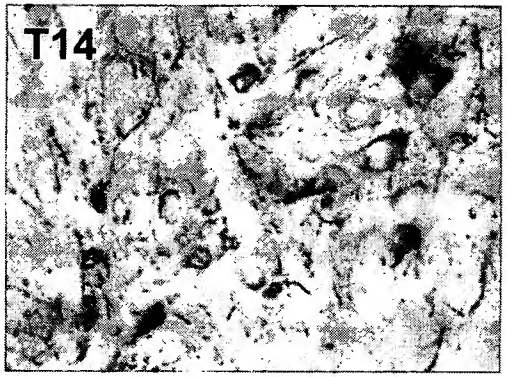


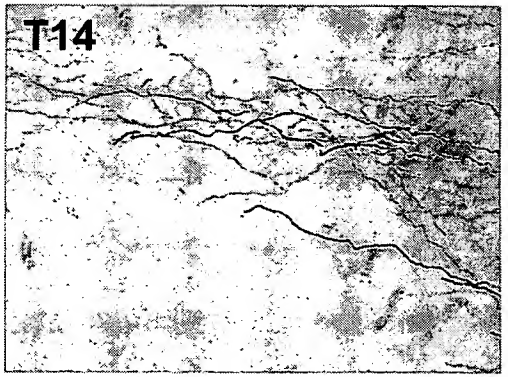


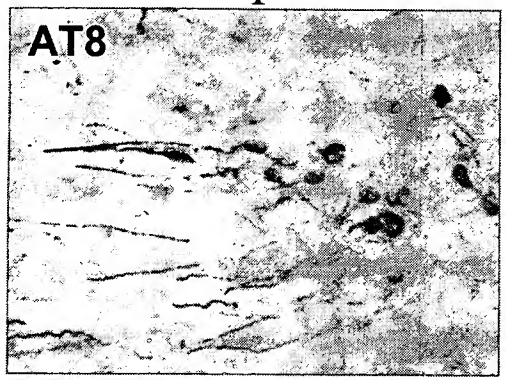
DGML – dentate gyrus molecular layer; NFT – neurofibrillary tangle; SLM- stratum lacunosum moleculare; SR – stratum radiatum

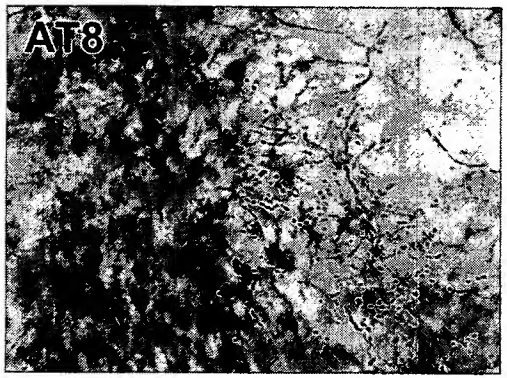
Histological Results

- Tau immunoreactivity was dense around the injection site in the entorhinal cortex and in perforant path efferents (T14 and AT8 immunolabeling).
- The presence of human tau vector product (T14) and hyperphosphorylation (AT8) appeared to be neuron-specific.



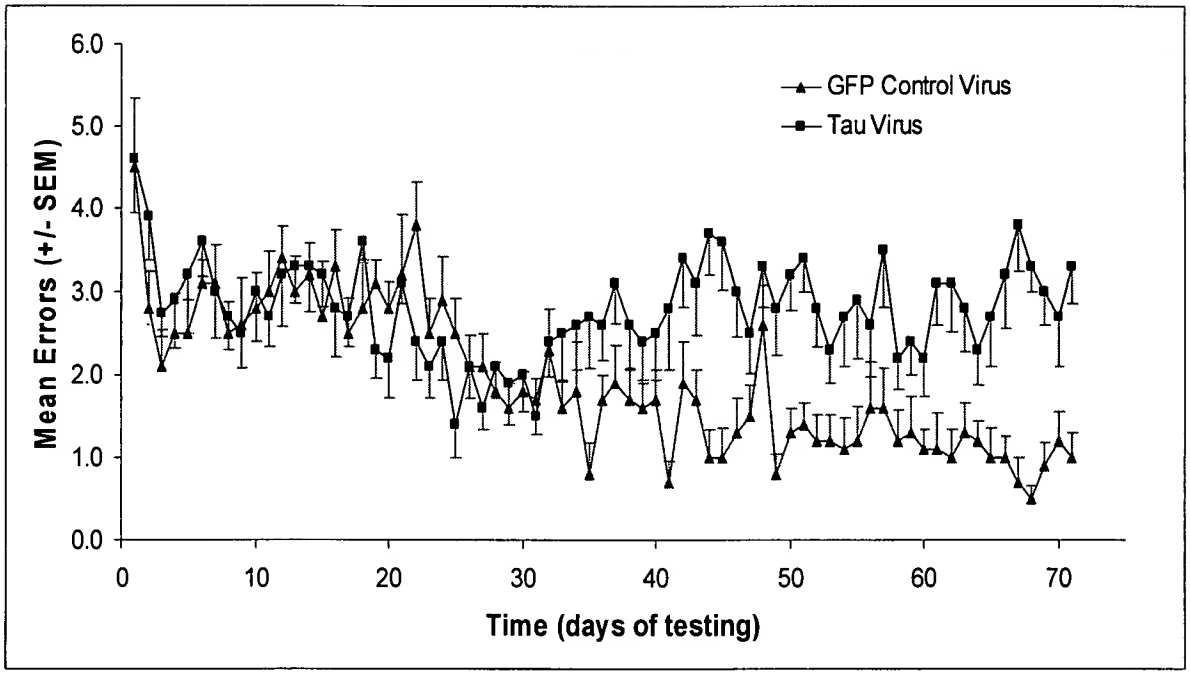






- The presence of AT8-positive neurons confirmed hyperphosphorylation of tau at ser202.
- The vector product was produced in both the somata and neurites of transduced neurons

Tau Expression Resulted in Mnemonic Deficits



- Tau vector-injected rats demonstrated significant spatial memory impairment relative to GFP vector-injected rats after the acquisition of the task.
- Although acquisition of the spatial alternation task was similar between the tau and the GFP vector-injected rats, the rats treated with the tau vector developed a severe impairment on the task after 30 days of testing (~1 month after intracerebral vector injection).

Major Findings

- Histological analysis revealed the presence of abnormal phosphorylation of tau and NFTs in the entorhinal cortex and neighboring retrohippocampal areas. Furthermore, degeneration was observed in the target regions of the perforant path.
- Transduction of entorhinal neurons with P301L tau virus vector significantly disrupted the working memory of rats performing a learned alternation task approximately one month after the intracerebral injection of the tau virus vector. Interestingly, the acquisition of the task was unimpaired.
- Given the histological results, the behavioral deficits seen in tau virus-injected rats are likely due to degeneration in and dysfunction of retrohippocampal structures, particularly the entorhinal cortex.
- Thus, an intracerebral injection of a virus vector constructed to drive the expression of mutated tau may be an excellent model of AD and other progressive disorders of the temporal lobe in which mutated tau may contribute to the mnemonic deficits.

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